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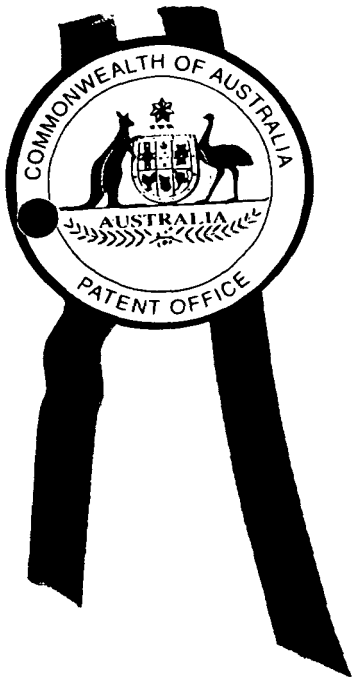
I, ANNA MAIJA MADL, ACTING TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PP 8073 for a patent by CSL LIMITED filed on 08 January 1999.



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Seventeenth day of February 2000

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Regulation 3.2

CSL Limited

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Patents Act 1990

PROVISIONAL SPECIFICATION
for the invention entitled:

"Improved immunogenic LHRH composition and methods relating thereto"

The invention is described in the following statement:

IMPROVED IMMUNOGENIC LHRH COMPOSITION AND METHODS RELATING THERETO

FIELD OF THE INVENTION

The present invention relates generally to an immunogenic LHRH composition having low reactogenicity, and more particularly to an immunogenic LHRH composition comprising a LHRH C-terminal fragment of at least five amino acids. The present invention is useful where the development of reactogenicity following administration of an LHRH composition is unacceptable, for example, as a prophylactic and/or therapeutic agent for the modification of fertility and behaviour patterns of domestic pets or livestock destined for consumption.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications referred to by author in this specification are collected at the end of the description.

Vaccination against the hypothalamic hormone luteinising hormone releasing hormone (referred to herein as "LHRH", also known as GnRH) has been demonstrated as an immunological method of controlling reproduction since the early 1970's (Fraser 1975, Jeffcoate *et al* 1982). Eliciting an immune response to LHRH prevents the release from the anterior pituitary of the hormones LH and FSH, which are required for the development and maintenance of the gonads - the testes in the male and ovaries in the female. Thus reduction of LH and FSH levels leads to loss of reproductive function.

De-sexing (or neutering) operations are the most widely practised surgical procedures in veterinary medicine and livestock animal management. A significant proportion of both sexes of domestic livestock and companion animals are routinely surgically de-sexed to prevent a variety of undesirable characteristics which accompany sexual maturity. The traits include fighting, wandering, sexual behaviour, loss of condition, tumours of reproductive organs and pregnancy.

The control of mating behaviour by vaccination with LHRH-conjugate vaccines in companion animals such as dogs, cats and horses, and in livestock specifically in male pigs and male and female cattle, has been identified as a goal as significant as the control of fertility.

Similarly, the control and treatment of disorders of the gonads and other reproductive organs, of both humans and animals, such as testicular cancer, breast cancer, prostate cancer, ovarian cancer, prostate enlargement or endometriosis is of significance.

The first published report of vaccination with an LHRH-conjugate vaccine in rabbits showed that a dramatic effect was achieved in the development of the testes. Early reports of the application of an LHRH vaccine in pigs (Falvo *et al*, 1986, Caraty and Bonneau 1986), showed that effective formulations based on 1-10 LHRH conjugated to human serum globulin or bovine serum albumin could control testes development and boar taint. Awonyi *et al.* (1988) showed that the effect of vaccination of pigs against LHRH affected primarily testis development.

The problems of variability of LHRH-conjugate vaccines in controlling boar taint have been attempted to be overcome by genetically incorporating LHRH amino acid sequences into carrier proteins, including the pilin gene from *E.coli* (Zee *et al* 1995) and into a truncated leucotoxin gene from *Pasteurella haemolytica* (Potter *et al* 1997). These fusion proteins are produced as recombinant molecules and not by biochemical coupling. Trials have shown these recombinant proteins to function as immunocastration vaccines. However, they have not resulted in commercially available vaccines and press reports suggest less than desired efficacy.

In keeping with the less than perfect nature of highly developed and widely applied subunit vaccines for disease prevention, immunocastration vaccines based on specific LHRH- protein conjugates have also been shown to be less than perfect at inducing antibody to LHRH or in reducing hormones or other parameters associated with reproductive functions. There has

been a general recognition of a wide variation in the effective induction of antibody to LHRH with a variety of LHRH-conjugate vaccines (Meloen *et al* 1994).

Vaccination of cattle with a 1-10 LHRH peptide - human serum albumin conjugate in Freunds adjuvant (Robertson *et al*, 1982), gave good antibody responses to LHRH after 2 vaccinations in only 5 of 10 vaccinated cattle. Even with boost vaccinations, the poor responders did not maintain antibody titres or have suppressed testosterone. A commercially developed vaccine for cattle (Vaxstrate), was only 80% effective (Hoskinson *et al* 1990).

Experiments in mice (Sad *et al* 1991) have shown that responses to LHRH-conjugates are genetically based. The vaccine was a 1-10 LHRH peptide, with the substitution of D-lysine instead of L-glycine at the 6 position, conjugated to diphtheria toxoid and adjuvanted with alum. Some strains of mice responded well, while other strains showed suppression of antibody to LHRH. These results would lead those skilled in the art of vaccine formulation to expect that a significant proportion of an outbred population would fail to respond or respond poorly to an LHRH-conjugate subunit type vaccine.

Vaccination of male pigs has resulted in variable suppression of testis development and suppression of boar taint. Bonneau and coworkers have shown (Bonneau *et al* 1994) that a 1-10 LHRH -α globulin conjugate given in oil emulsion for primary vaccination and saponin adjuvant for boost vaccination gave an antibody response in only 90% of 20 vaccinated pigs. Testosterone levels were suppressed in only 16/20 vaccinates (75%). Thus the quality as well as the amount of antibody is important in determining the efficacy of an LHRH-conjugate based vaccine. Hagen *et al* (1988) claimed that vaccination of 6 boars with an LHRH-bovine serum albumin (BSA) conjugate in Freunds adjuvant could reduce boar taint. However, 2/6 boars had low antibody responses and had normal spermatogenesis and testis function. Skatole levels were not affected by vaccination against LHRH.

It has been determined that the efficacy of vaccination against LHRH is significantly improved when LHRH is administered as a conjugate with diphtheria toxoid and an ionic

polysaccharide such as DEAE-dextran. However, although the efficacy is improved, this conjugate nevertheless induces reactogenicity such as visible swelling, that may be long-lasting, at the site of administration. In some instances, such as where the conjugate is used to vaccinate domestic pets (for example, dogs) or livestock destined for consumption the occurrence of such reactogenicity following vaccination against LHRH utilising this conjugate is unacceptable. Accordingly, there is a need to develop a LHRH vaccine which exhibits both efficacy and low reactogenicity.

In work leading up to the present invention, it has been determined that reactogenicity of LHRH conjugated to diphtheria toxoid and an ionic polysaccharide is reduced when a proportion of the ionic polysaccharide component is replaced with an immunostimulating complex.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

Sequence Identity Numbers (SEQ ID NOS.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography. A summary of the SEQ ID NOS. is provided after the Examples.

One aspect of the present invention relates to a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and an immunostimulating complex component wherein the reactogenicity of said preparation is low.

Still another aspect of the present invention there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and a protein-free immunostimulating complex component wherein the reactogenicity of said preparation is low.

In yet another aspect there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and an immunostimulating complex component where the reactogenicity of said preparation is low.

In still yet another aspect there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and a protein-free immunostimulating complex component, wherein the reactogenicity of said preparation is low.

In a further aspect of the present invention there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a modified LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and an immunostimulating complex component wherein the reactogenicity of said preparation is low.

In another further aspect there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a modified LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and a protein-free immunostimulating complex component, wherein the reactogenicity of said preparation is low.

Preferably, the ionic polysaccharide component is a DEAE-dextran component.

In still another further aspect of the present invention there is provided a pharmaceutical composition comprising a LHRH-diphtheria-toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and an immunostimulating complex component, together with one or more pharmaceutically acceptable carriers and/or diluents.

In still yet another further aspect of the present invention there is provided a pharmaceutical composition comprising a LHRH-diphtheria-toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and a protein-free immunostimulating complex component, together with one or more pharmaceutically acceptable carriers and/or diluents.

Another aspect of the present invention relates to a method of eliciting, in an animal, an effective immune response to LHRH, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Still another aspect of the present invention relates to a method of eliciting, in an animal, an effective immune response to LHRH which inhibits the reproductive capacity of said animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Yet another aspect of the present invention relates to a method of castrating an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation where the reactogenicity of said preparation is low.

Still yet another aspect of the present invention relates to a method of regulating oestrus cycling in a female animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

A further aspect of the present invention relates to a method of inhibiting characteristics induced by the sexual maturation of an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In another further aspect there is provided a method of inhibiting aggression in an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In still another further aspect there is provided a method of inhibiting sexual activity in an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In yet another further aspect there is provided a method of inhibiting behavioural and/or physiological characteristics induced by the sexual maturation of cats and/or dogs, said method comprising administering to said cat and/or dog an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In still yet another further aspect there is provided a method of inhibiting behavioural and/or physiological characteristics induced by the sexual maturation of horses, said method comprising administering to said horse an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Another aspect of the present invention relates to a method of achieving production gains in livestock, said method comprising administering to said livestock an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In still another aspect of the present invention relates to a method of inhibiting the growth of cells which are regulated directly or indirectly by LHRH, said method comprising

administering an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In still yet another aspect of the present invention there is provided a method of down-regulating the libido of an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

References herein to "LHRH-conjugate preparation" are to be understood as references to the LHRH preparation of the present invention as broadly described above.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is predicated, in part, on the determination that when a proportion of the ionic polysaccharide component of LHRH conjugated to diphtheria toxoid and an ionic polysaccharide is replaced with an immunostimulating complex, the reactogenicity of the LHRH conjugate is reduced without significant reduction in efficacy. Development of this novel LHRH conjugate preparation permits vaccination against LHRH in circumstances where the development of reactogenicity (for example, at the site of administration) is not acceptable.

Accordingly, one aspect of the present invention relates to a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and an immunostimulating complex component wherein the reactogenicity of said preparation is low.

Reference to an "ionic polysaccharide" should be understood as a reference to any positively or negatively charged polysaccharide or derivative or chemical equivalent thereof. Reference to "derivative" and "chemical equivalent" should be understood to have the same meaning as

outlined below. Said ionic polysaccharide may be in soluble or insoluble form. Preferably said ionic polysaccharide is an ionic dextran. Even more preferably said ionic dextran is DEAE-dextran, dextran sulphate or QAE-dextran. Most preferably, said ionic dextran is DEAE dextran. Preferably, the dextran component of said ionic dextran exhibits a molecular weight in the range 250,000 to 4,000,000 Da and even more preferably 500,000 to 1,500,000 Da.

Accordingly, the present invention more particularly provides a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises a DEAE-dextran component and an immunostimulating complex component wherein the reactogenicity of said preparation is low.

The development of reactogenicity to a preparation, such as at the site of administration of a vaccine, is usually assessed by reference to the development of a number of symptoms including symptoms of inflammation such as swelling which is detectable either by palpation or, if more severe, by the eye, redness, and abscess formation. The degree of reactogenicity is usually determined by reference to the occurrence, duration and severity of any one or more of these symptoms. For example, reactogenicity which results in visible abscess formation is of greater severity than reactogenicity which involves only swelling. Further, swelling which is visible to the eye is a more severe form of reactogenicity than swelling which is detectable only by palpation. In accordance with the method of the present invention, reference to reactogenicity which is "low" should be understood as reactogenicity which produces either no detectable symptoms or symptoms which are not visible to the eye. For example, swelling which is detectable only by palpation is an example of reactogenicity which is low. The method of the present invention should be understood to extend to the complete absence of any reactogenicity. In the context of the present invention, low reactogenicity may also be taken to include visible swelling of only short duration.

An immunostimulating complex (or Iscom™) which is incorporated in a preparation in accordance with the present invention may be prepared by techniques which are well known to persons skilled in the art, and which are described in detail in the publications of Cox and Coulter, 1992 and Morein *et al.*, Australian Patent Specifications No. 558258, 589915, 590904 and 632067 the disclosures of which are incorporated by reference herein.

Briefly, immunostimulating complexes are typically, but not limited to, small cage like structures 30-40nM in diameter. The final formulation of an immunostimulating complex with an optimal amount of protein is a molar ratio of Quil A, cholesterol, phosphatidyl choline and protein in a ratio of 1:1:1:1. An immunostimulating complex may contain, for example, 5 to 10% by weight Quil A, 1 to 5% cholesterol and phospholipids and the remainder protein. Peptides can be incorporated into the immunostimulating complex either directly or by chemical coupling to a carrier protein (e.g. diphtheria toxin or influenza envelope protein) after incorporation of protein into immunostimulating complexes. Reference to an "immunostimulating complex" should be understood to include reference to derivatives, chemical equivalents and analogues thereof. For example, reference to a derivative of an immunostimulating complex includes reference to an immunostimulating complex in which one or more of Quil A, cholesterol, phosphatidyl choline or protein, for example, are deleted or where a component in addition to Quil A, cholesterol, phosphatidyl choline or protein is added to the complex. The functional equivalent of an immunostimulating complex may be an immunostimulating complex in which one or more of its four components are replaced with a functional equivalent. In a preferred embodiment of the present invention, the protein component of the immunostimulating complex is deleted. This type of immunostimulating complex is herein referred to as a "protein-free immunostimulating complex" (or Iscomatrix™).

According to this embodiment of the present invention there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH diphtheria toxoid conjugate adsorbed to an adjuvant which adjuvant comprises an ionic

polysaccharide component (preferably a DEAE-dextran component) and a protein-free immunostimulating complex component wherein the reactogenicity of said preparation is low.

Reference to an "effective" immune response should be understood as a reference to an immune response which either directly or indirectly leads to the reduction or complete blocking of reproductive function (i.e. reduces or prevents the development of or functioning of any one or more of the reproductive organ's activities or modulates the hormonal levels of an animal such that any one or more aspects of reproduction or reproductive activity are reduced) in at least 90%, and preferably at least 95%, of animals treated. It should be understood that efficacy is a functional measure and is not defined by reference to anti-LHRH antibody titre alone since the presence of circulating antibody alone is not necessarily indicative of the capacity of said circulating antibody to block reproductive function. The term "reproductive organ" should be understood in its broadest sense to refer to the male and female gonads and accessory sexual organs. "Accessory sexual organs" should also be understood in its broadest sense and includes, for example, the prostate, breasts, seminal vesicles and the uterus.

Reference hereinafter to "LHRH" should be read as including reference to all forms of LHRH and derivatives, equivalents and analogues thereof.

Reference to "derivatives, equivalents and analogues" should be understood to include reference to fragments, parts, portions, chemical equivalents, mutants, homologues and analogues from natural, synthetic or recombinant sources, including fusion proteins. For example, with respect to LHRH, said LHRH includes peptides comprising a sequence of amino acids substantially as set forth in SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or having at least 50% similarity thereto. The molecules defined in SEQ ID Nos:1, 2 and 3 are from the human and are conserved across all mammals. SEQ ID NO:4 is a derivative of SEQ ID NO:2 wherein spacers have been introduced at the N-terminus. Chemical equivalents of LHRH can act as a functional analogue of LHRH. Chemical equivalents may not necessarily be derived from LHRH but may share certain similarities.

Alternatively, chemical equivalents may be specifically designed to mimic certain physiochemical properties of LHRH. Chemical equivalents may be chemically synthesised or may be detected following, for example, natural product screening.

Homologues of LHRH contemplated herein include, but are not limited to, LHRH derived from different phyla including birds, fish, reptiles and invertebrates.

"Derivatives" may also be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acids sequence variants are those in which one or more amino acid or non-natural amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more amino acids from sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different natural or non-natural residue inserted in its place. Typical substitutions are those made in accordance with Table 1:

TABLE 1

Suitable residues for amino acid substitutions

	Original Residue	Exemplary Substitutions
	Ala	Ser
*	Arg	Lys
	Asn	Gln; His
	Asp	Glu
	Cys	Ser
	Gln	Asn
*	Glu	Ala
*	Gly	Pro
*	His	Asn; Gln
	Ile	Leu; Val
*	Leu	Ile; Val
	Lys	Arg; Gln; Glu
	Met	Leu; Ile
	Phe	Met; Leu; Tyr
*	Ser	Thr
	Thr	Ser
*	Trp	Tyr
*	Tyr	Trp; Phe
	Val	Ile; Leu

Key: Amino acid residues marked with an asterisk indicate residues present in the mammalian LHRH sequence.

Examples of non-natural amino acids include, but are not limited to the D-isomers of said amino acids. "Additions" to amino acid sequences include fusion with other peptides, polypeptides or proteins.

Reference to diphtheria toxoid should be understood as a reference to all forms of diphtheria toxoid and derivatives thereof. The term "derivatives" has the same meaning as hereinbefore defined. Derivatives may include, for example, molecules comprising the diphtheria toxoid T cell epitopes or said T cell epitopes in isolation.

Preferably, said LHRH comprises an LHRH C-terminal fragment of at least five amino acids. More preferably, said LHRH is full length LHRH or "LHRH 1-10 form" which comprises the amino acid sequence substantially as set forth in SEQ ID NO:1. Even more preferably, said LHRH comprises the amino acid sequence substantially as set forth in SEQ ID NO:2 and wherein the carboxyl terminus of said amino acid sequence is amidated. Said preferred LHRH is referred to herein as "LHRH 2-10 form".

Accordingly, in one preferred embodiment, there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, an ionic polysaccharide component (preferably which adjuvant comprises a DEAE-dextran component) and an immunostimulating complex component wherein the reactogenicity of said preparation is low.

In another embodiment, there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component (preferably a DEAE-dextran component) and a protein-free immunostimulating complex component, wherein the reactogenicity of said preparation is low.

In another preferred embodiment said LHRH comprises the amino acid sequence substantially as set forth in SEQ ID NO:4. Said preferred LHRH is referred to herein as "modified LHRH 2-10 form".

In yet another embodiment of the present invention there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a modified

LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component (preferably a DEAE-dextran component) and an immunostimulating complex component wherein the reactogenicity of said preparation is low.

In yet another preferred embodiment there is provided a preparation for use in eliciting an effective immune response to LHRH, said preparation comprising a modified LHRH 2-10 form-diphtheria toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component (preferably a DEAE-dextran component) and a protein-free immunostimulating complex component, wherein the reactogenicity of said preparation is low.

Although not intending to limit the invention to any one method, said peptide may be synthesised by Fmoc chemistry and the resulting peptide coupled to the carrier protein diphtheria toxoid. Said coupling may be performed as described in Ladd *et al* 1990 or in Bonneau *et al* 1994, and the resulting conjugate of peptide and carrier protein (referred to herein as "peptide-conjugate") processed to be free of unbound peptide and other by-products of conjugation. Such processing may be achieved by conventional dialysis or by ultrafiltration. The resulting peptide-conjugate is adsorbed to the ionic polysaccharide adjuvant.

Still without limiting the present invention to any one theory or mode of action, administration of an effective amount of the LHRH preparation of the present invention induces a significantly more effective immune response against LHRH than the LHRH-carrier-adjuvant compositions described in the prior art. Said improved efficacy is observed when the immunogenic LHRH composition specifically comprises the carrier diphtheria-toxoid and an ionic polysaccharide adjuvant. By replacing a portion of the ionic polysaccharide component with an immunostimulating complex component, the reactogenicity of the LHRH-conjugate preparation can be reduced while maintaining its efficacy.

LHRH-conjugate preparations suitable for use in accordance with the present invention preferably comprise 5-500μg of LHRH-diphtheria toxoid conjugate in 5-500 μg ionic polysaccharide (such as DEAE-dextran)/10-2000 μg immunostimulating complex (such as Iscomatrix™).

In another aspect of the present invention there is provided a pharmaceutical composition comprising a LHRH-diphtheria-toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and an immunostimulating complex component, together with one or more pharmaceutically acceptable carriers and/or diluents.

In yet another aspect of the present invention there is provided a pharmaceutical composition comprising a LHRH-diphtheria-toxoid conjugate adsorbed to an adjuvant, which adjuvant comprises an ionic polysaccharide component and a protein-free immunostimulating complex component, together with one or more pharmaceutically acceptable carriers and/or diluents.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or salts such as sodium chloride. Prolonged absorption or delayed release of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying, the freeze-drying technique and the spray-drying technique which yield a powder of the active ingredients plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or compressed into tablets, or incorporated directly with the food of the diet. For oral administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1μg and 2000μg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: a binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; and a lubricant such as magnesium stearate. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. A syrup or elixir may contain the active compound, methyl and propylparabens as preservatives, and a dye. Of course, any material used in preparing any dosage unit form should be

veterinarily pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for veterinarily active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. For administration to livestock it is particularly advantageous to use a multi-dose container linked to a repeating vaccination gun. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μg to about 2000 μg. Expressed in proportions, the active compound is generally present in from about 0.5 μg to about 2000 μg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

Although not intending to limit the invention to any one theory or mode of action, the induction of an effective immune response against LHRH results in prevention of the release of the hormones LH and FSH from the anterior pituitary. Since these hormones are required for the development and maintenance of the gonads, reduction in the levels of these hormones leads to a decrease or loss of reproductive functions. The vaccinated animals are therefore effectively neutered resulting in the loss of characteristics associated with sexual maturity such as fighting, wandering, sexual behaviour, loss of condition, organoleptic characteristics, tumours of reproductive organs and pregnancy.

Accordingly, another aspect of the present invention relates to a method of eliciting, in an animal, an effective immune response to LHRH, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Reference to "LHRH-conjugate preparation" should be understood as a reference to the LHRH preparation of the present invention as broadly described above.

Reference to "animal" should be understood as the reference to all animals including primates (e.g. humans, monkeys), livestock animals (e.g. sheep, cows, horses, donkeys, goats, pigs), laboratory test animals (e.g. rats, guinea pigs, rabbits, hamsters), companion animals (e.g. dogs, cats), captive wild animals (e.g. emus, kangaroos, deer, foxes), aves (e.g. chickens, ducks, bantams, pheasants, emus, ostriches), reptiles (e.g. lizards, snakes, frogs) and fish (e.g. trout, salmon, tuna). Said animal may be male or female.

In a most preferred embodiment the present invention relates to a method of eliciting, in an animal, an effective immune response to LHRH, which inhibits the reproductive capacity of said animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Reference to "inhibiting the reproductive capacity of an animal" should be understood as the partial or complete reduction of the functioning of any one or more of the reproductive organs's activities or modulation of said animal's hormonal levels such that reproductive activity, such as sexual activity, is reduced.

Inhibiting the reproductive capacity of an animal may result in a number of consequences such as, but not limited to, the castration of said animal or the reduction or elimination of characteristics associated with sexual maturity (for example, fighting, wandering, sexual behaviour, loss of condition, unwanted organoleptic characteristics, tumours of reproductive organs and pregnancy). "Castration" should be understood as a reference to the neutering of both male and female animals. Inhibiting the reproductive capacity of an animal may also result in the cessation of tumor cell proliferation (for e.g. prostate cancer cells, breast cancer cells, ovarian cancer cells or testicular cancer cells), inhibition or reversal of hyperplasia, such as prostate enlargement, endometriosis or inflammatory responses.

Accordingly, another aspect of the present invention relates to a method of castrating an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Yet another aspect of the present invention relates to a method of regulating oestrus cycling in a female animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Reference to "regulating" should be understood in its broadest sense and includes, for example, inhibiting or delaying oestrus.

Still yet another aspect of the present invention relates to a method of inhibiting characteristics induced by the sexual maturation of an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Reference to "inhibiting characteristics induced by the sexual maturation of an animal" should be understood as a reference to the reduction or complete elimination of any one or more physical and/or behavioural characteristics induced either directly or indirectly by sexual maturation. Said physical and/or behavioural characteristics include, for example, fighting, wandering, sexual behaviour, loss of condition, unwanted organoleptic characteristics, oestrus cycling, fertility, pregnancy and tumours of the reproductive organs. Accordingly, inhibiting said characteristics includes inhibiting sexual activity (for example preventing female cattle mounting other female cattle) preventing or delaying ovulation, reducing aggressive behaviour or reducing unwanted organoleptic characteristics such as boar taint. In a particularly preferred embodiment, said characteristics are aggression and sexual activity.

According to this preferred embodiment there is provided a method of inhibiting aggression in an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

In another preferred embodiment there is provided a method of inhibiting sexual activity in an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Vaccination with LHRH in male dogs and cats can be used to control unwanted behaviour, particularly aggression and the urge to roam. In female dogs and cats, the desired effects are control of fertility and of unwanted behaviour at the time of oestrus, commonly termed "in heat" or "in season". The unwanted behaviour in females includes increased fractiousness, marking of territories, wandering and other behaviours associated with oestrus. However, in particular with domestic pets, the occurrence of reactogenicity at the site of vaccination, such as persistent swellings or in extreme cases abscess formation, is unacceptable to the pet owner.

According to this most preferred embodiment there is provided a method of inhibiting characteristics induced by the sexual maturation of cats and/or dogs, said method comprising

administering to said cat and/or dog an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Most preferably said characteristics are aggression and roaming in male cats and/or dogs and fractiousness, marking of territory, wandering and oestrus behaviour in female cats and/or dogs.

In the thoroughbred horse industry, the racing of stallions is associated with difficulty in handling and ease of training and consistency of performance. A large proportion of young colts are gelded and raised as castrates to make them more manageable. This does not appear to impact significantly on their racing potential. A vaccine to control unwanted behavioural problems would allow the full racing potential of male horses to be realised, with the added benefit of possible reversibility and so obtaining the genetic benefit as a stud animal after their racing career is over.

The racing of fillies and mares (female horses) is at its height in the spring and to some degree in the autumn in the temperate climates of the world. It is at these times of the year that horses come into season. This causes difficulties in training, handling and in uneven and poor racing performance. An LHRH vaccine to control oestrus would have a large and ready market in the horse racing industry. There are currently products based on hormone analogues available to control oestrus in racing fillies and mares. These are reported to have a lasting effect on the ability of treated mares to breed.

Accordingly, in yet another preferred embodiment there is provided a method of inhibiting characteristics induced by the sexual maturation of horses, said method comprising administering to said horse an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Most preferably said characteristics are aggression in colts and oestrus behaviour and uneven performance in mares.

In cattle, the unmanageable behaviour of bulls is well known. Aggression of bulls can be directed toward stockmen, inanimate objects such as fences and drinking troughs and can result in serious fighting between cattle. Thus in most beef producing countries, bulls destined for beef production are castrated while still calves, and the resulting steers are raised. The raising of steers in preference to entire males has a significant negative impact on production performance, but this is judged to be an acceptable, even necessary trade off over the raising of more docile steers.

Heifers are raised for beef production in the USA and in Australia. The cycling of heifers in feedlots causes significant production losses. The cycling heifer has a large increase in activity levels, resulting in poor or negative growth over the 5-7 days of the cycle. The heightened activity levels of heifers in oestrus impacts on other heifers in the same pen, so that the production performance of the entire pen of 50-100 animals is affected. In the USA heifers are fed a diet containing melengestrol acetate (MGA), a synthetic steroid, to control oestrus. In Australia, and other countries where hormonal feed supplements are prohibited, heifers are raised in feedlots without feeding of MGA, with poor production performance.

Accordingly, the immunocastration of livestock, although reducing or eliminating characteristics associated with sexual maturity, generally results in a negative impact on production gains on immunocastrated animals over uncastrated animals. This theory is based on the well established fact that entire animals grow considerably faster and more efficiently than castrated animals. It has been determined that administering the LHRH preparation of the present invention to livestock nevertheless results in the achievement of production gains. Reference herein to "production gains" includes but is not limited to an increase in final weight of livestock at slaughter, lowering of feed requirements for each kilogram of carcass weight gained, increasing growth rate of said livestock as compared to uncastrated livestock, improving the quality of meat derived from said livestock (for example, by controlling unwanted organoleptic characteristics of said meat) or decreasing stress levels of intensively housed livestock by reducing aggressive interactions of the intensively housed animals or, with respect to pigs, control of boar taint. However, the occurrence of reactogenicity in

animals destined for slaughter and consumption is not desirable due to its unsightly appearance and the losses involved in trimming the carcase for consumption. Accordingly, the use of a preparation which minimises the incidence and severity of reactogenicity is highly desirable.

Accordingly, yet another aspect of the present invention relates to a method of achieving production gains in livestock, said method comprising administering to said livestock an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

The LHRH-conjugate preparation may be administered to the livestock in a single-dose, for example a single administration of a slow or pulsatile release vaccine or in multiple doses.

The term "livestock" includes but is not limited to mammals such as pigs, cattle, sheep; captive wild animals such as deer; and aves such as emus or ostriches. Most preferably, said livestock are cattle.

In animals, and particularly humans, vaccination with a LHRH-conjugate preparation can be used as a prophylactic or therapeutic treatment for disorders which are modulated directly or indirectly by LHRH. The incidence of low reactogenicity when using this preparation is particularly desirable when the subject being treated is a human. The disorders which are the subject of treatment include malignancies of cells which are regulated by LHRH or regulated by hormones which are themselves regulated by LHRH, for example, testicular cancer, breast cancer, ovarian cancer, prostate cancer and cancers of oncofoetal cells or cells which are induced to express oncofoetal antigens when malignancy occurs. These disorders also include non malignant proliferative disorders such as hyperplasias, for example, prostatic hyperplasia or endometrial hyperplasia. Without limiting the present invention to any one theory or mode of action, some tumor cells express receptors for reproductive hormones, the synthesis of which are regulated by LHRH. By vaccinating against LHRH it is possible to prevent the release of these hormones. The LHRH-conjugate preparation of the present invention may also be used to treat or prevent disorders such as ovarian polycystitis, endometriosis and

inflammatory conditions. Further uses of the LHRH-conjugate preparation of the present invention include human fertility treatment based on modulation of the libido.

Accordingly, another aspect of the present invention relates to a method of inhibiting the growth of cells which are regulated directly or indirectly by LHRH, said method comprising administering an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Preferably said cells are human cells.

Reference to cell "growth" is a reference to the proliferation, differentiation or functional activity of said cell. Reference to cell growth which is "regulated directly or indirectly by LHRH" should be understood as a reference to cell growth which is regulated by LHRH itself or cell growth which is regulated by hormones other than LHRH which are themselves either directly or indirectly regulated by LHRH.

Reference to "inhibiting" should be understood as a reference to the prevention of cell growth, the cessation of cell growth or the down regulation of cell growth. Said cells may be located within the organ from which they derive or at some other location within the animal's body, such as, for example, where a malignant breast cell has metastasised in the liver.

In a particularly preferred embodiment said cells are malignant cells and most particularly malignant testicular cells, malignant breast cells, malignant ovarian cells or malignant prostate cells.

In yet another preferred embodiment said cells are hyperplastic cells such as prostatic hyperplastic cells or endometrial hyperplastic cells.

In yet another aspect of the present invention there is provided a method of down-regulating the libido of an animal, said method comprising administering to said animal an effective amount of LHRH-conjugate preparation wherein the reactogenicity of said preparation is low.

Preferably said animal is a human.

Further features of the present invention are more fully described in the following Examples. It is to be understood, however, that this detailed description is included solely for the purposes of exemplifying the present invention. It should not be understood in any way as a restriction on the broad description of the invention as set out above.

EXAMPLE 1
PREPARATION OF LHRH-CONJUGATE PREPARATION

The LHRH-conjugate is based on a synthetic 2-10 form of Luteinising Hormone Releasing Hormone (LHRH) peptide coupled to a carrier protein. The peptide by itself is too small to be antigenic, and coupling to a carrier protein is required so that the peptide acts as a hapten and immunity is induced to LHRH. The carrier protein is diphtheria-toxoid.

The peptide is synthesised by Fmoc chemistry and the resulting 2-10 form LHRH peptide is coupled to diphtheria toxoid. The coupling may be performed as described in Ladd et al. 1990 or in Bonneau et al. 1994, and the resulting conjugate of peptide and diphtheria-toxoid processed to be free of unbound peptide and other by-products of conjugation. Such processing may be achieved by conventional dialysis or by ultrafiltration.

The resulting LHRH-carrier protein preparation may be used to prepare a composition for administration by formulation with or in an adjuvant (referred to as "LHRH-conjugate preparation "). The adjuvant is an ionic polysaccharide such as DEAE-dextran, dextran sulphate or QAE-dextran. The adjuvant formulation may include a combination of two or more of the adjuvants listed. These lists are not to be taken as exhaustive. The selection of

adjuvant is in part dependent on the species being targeted and is based on the level and duration of the immune response required and on the lack of reactogenicity (ie tissue compatibility). The level of active component and adjuvant are chosen to achieve the desired level and duration of immune response.

EXAMPLE 2

PREPARATION OF PROTEIN-FREE IMMUNOSTIMULATING COMPLEX (ISCOMATRIX™)

Quil A solution (sterile) and dipalmitoyl phosphatidyl choline (DPPC)/cholesterol solution in 20% Mega 10 detergent are aseptically combined in a sterile temperature controlled reaction vessel in quantities calculated to result in starting proportions of 5:1:1 for QuilA:DPPC:cholesterol (by weight). After reaction, the preparation is ultrafiltered to remove unincorporated starting materials and free Mega 10 detergent. This preparation, known as Iscomatrix™, is essentially a preformed adjuvant consisting of protein-free immunostimulating complex particles.

EXAMPLE 3

PREPARATION OF VACCINES FOR DOG TRIALS

A. DEAE-dextran adjuvant alone.

The formulation required to be tested in this trial was a 1mL dose containing 5-200 mg DEAE dextran, 5-500 μg LHRH-diphtheria toxoid conjugate, with thiomersal (0.01%) added as preservative, in sterile phosphate buffered saline.

The adjuvant for this vaccine is prepared by dissolving DEAE-dextran (Pharmacia, Uppsala, Sweden, Molecular Weight average >500,000) in distilled water and adjusting the pH to 7.5

with hydrochloric acid. The final concentration of the DEAE-dextran is adjusted to 20% w/v by the addition of distilled water, after pH adjustment. The solution is then sterile filtered. LHRH peptide (2-10 LHRH) is conjugated to diphtheria toxoid as described in Example 1. The conjugate preparation is sterile filtered and the protein concentration determined by standard methods, e.g. the BCA protein assay. The preparation used for this study was 5.7 mg protein/mL.

B. Iscomatrix™ adjuvant alone.

The formulation required to be tested in this trial was a 1mL dose containing 10-1000 μg Iscomatrix™, 5-500 μg LHRH-diphtheria toxoid conjugate, with thiomersal (0.01%) added as preservative in sterile phosphate buffered saline.

The adjuvant for this vaccine, Iscomatrix™, is prepared as described in Example 2 using Quil A as the saponin derivative, together with cholesterol, dipalmitoyl phosphatidyl choline (DPPC) and Mega 10 as the detergent. The process is performed under sterile conditions with all components sterilised prior to addition. The Quil A concentration was determined to be 2.24mg/ml.

LHRH peptide (2-10 LHRH) is conjugated to diphtheria toxoid as described in Example 1. The conjugate preparation is sterile filtered and the protein concentration determined by standard methods, e.g. the BCA protein assay. The preparation used for this study was 5.7 mg protein/mL.

C. Iscomatrix™ + DEAE dextran

The formulation required to be tested in this trial was a 1mL dose containing 10-1000 μg Iscomatrix™ + 5-200 mg DEAE-dextran, 5-500 μg LHRH-diphtheria toxoid conjugate, with thiomersal (0.01%) added as preservative, in sterile phosphate buffered saline.

The adjuvant for this vaccine is a compound adjuvant, obtained by combining appropriate proportions of Iscomatrix™ and DEAE-dextran. Iscomatrix™, is prepared as described in Example 2. DEAE-dextran is prepared as described in Example 3A above.

LHRH peptide (2-10 LHRH) is conjugated to diphtheria toxoid as described in Example 1. The conjugate preparation is sterile filtered and the protein concentration determined by standard methods, e.g. the BCA protein assay. The preparation used for this study was 4.9 mg protein/mL.

EXAMPLE 4
VACCINATION OF DOGS

All trials were conducted in an identical manner.

Dogs of 6-12 months of age and of mixed sex were housed in indoor pens with free access to outdoor runs. They were fed a commercially available balanced diet.

Dogs were vaccinated subcutaneously in the scruff of the neck at 0 and 4 weeks with a 1 mL dose of vaccine. All vaccines were administered from a 2 mL syringe fitted with a 23 gauge needle.

Dogs were bled at regular intervals to monitor antibody levels to LHRH.

Reactogenicity of the vaccines was determined by close examination of the dogs after vaccination.

Examination was by visual inspection and by thorough manual palpation of the vaccine site and surrounding area.

Site reactions were scored by close visual examination and physical palpation of the injection site and surrounding area. Reactions were scored as:

0 No reaction

1 Lump, not visible, only detected by palpation

2 Swelling, readily detectable by visual examination

3 Abscessed swelling, detectable by visual examination

SITE REACTION SCORES

Vaccine Formulation	1 Week PB			2 Weeks PB			4 Weeks PB		
	0	1	2	0	1	2	0	1	2
DEAE-dextran	3/7	2/7	2/7	3/7	4/7	-	7/7	-	-
Iscomatrix™	7/7	-	-	7/7	-	-	7/7	-	-
DEAE-dextran + Iscomatrix™	5/7	1/7	1/7	7/7	-	-	7/7	-	-

(Note: PB = post-boost)

These results show that the DEAE dextran formulation is much more reactive than either the Iscomatrix™ formulation or the Iscomatrix™ +DEAE-dextran formulation. With DEAE-dextran alone, significant reactions (Score 1 or greater) persisted for at least 2 weeks. With the Iscomatrix™ formulation, reactions were not detected and with the DEAE-dextran + Iscomatrix™ formulation, minor reactions in 2/7 dogs persisted for only 1 week.

In order to provide a more quantitative indication of reactogenicity, the approximate volumes of the site reactions, when detected, were measured as width x length x height, in cm. The results for 1 and 2 week post-boost (PB)from the above table are given below. Site reaction volumes are not given for the Iscomatrix™ vaccine formulation group, as no site reactions were detected.

SITE REACTION VOLUMES

Vaccine Formulation	Week 1 PB Site Reaction Volumes (cm^3)			Week 2 PB Site Reaction Volumes (cm^3)		
	Score 0	1	2	Score 0	1	2
DEAE-dextran	-	32, 4	40, 8	-	6.25,1 0.5,0.5	-
Iscomatrix™ + DEAE-dextran		1.1	6	-	-	-

These results show that the degree of reactogenicity is much less in the Iscomatrix™ + DEAE-dextran formulation than with the DEAE-dextran formulation alone as shown by the size of the reactions.

The following antibody data provide evidence of the efficacy of the vaccine formulations. Antibody titres to LHRH are given at 8 weeks post boost to better indicate the duration of the antibody response.

ANTIBODY TITRES TO LHRH (8 weeks post-boost)

Vaccine Formulation	Geometric mean titre	Range
DEAE-dextran	38,913	23,166 - 50,377
Iscomatrix™	3,965	3,322 - 7,343
DEAE-dextran + Iscomatrix™	9,729	4,149 - 35,112

These results show that the formulation based on DEAE-dextran + Iscomatrix™ gives comparable titres to DEAE-dextran alone, particularly when comparing the range of titres obtained, while also giving a significant decrease in the degree of reactogenicity. In addition, the DEAE-dextran + Iscomatrix™ formulation gave increased antibody titre when compared to the Iscomatrix™ formulation, when the same levels of LHRH-conjugate and Iscomatrix™ were used in the two formulations.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: CSL LIMITED

(ii) TITLE OF INVENTION: IMPROVED IMMUNOGENIC LHRH COMPOSITIONS AND METHODS RELATING THERETO

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
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(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

p-Glu His Trp Ser Tyr Gly Leu Arg Pro Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

His Trp Ser Tyr Gly Leu Arg Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Trp Ser Tyr Gly Leu Arg Pro Gly
1 5

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Ser Gly Ser Gly Leu Arg Pro Gly
1 5

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